lymphoma biology

390 ONCOGENIC ASSOCIATION OF THE CBP/PAG ADAPTOR PROTEIN WITH THE LYN TYROSINE KINASE IN HUMAN B-NHL RAFTS

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To control proliferation and apoptosis, B-non-Hodgkin lymphomas utilize a plasma membrane, raft-associated signalosome made of the constitutively active Lyn kinase, the tyrosine phosphorylated Cbp/PAG adaptor and tyrosine phosphorylated STAT3 transcription factor. No such signalosome is found in rafts of ALK+ T-lymphoma and Hodgkin-derived cell lines, despite similar Cbp/PAG, Lyn and STAT3 expression, and similar amounts of raft sphingolipids. We suggest that stable association of the signalosome with B-NHL rafts requires (i) a Lyn kinase (auto)phosphorylated in its regulatory and active site tyrosines, (ii) a Cbp/PAG adaptor phosphorylated at tyrosine 317 and bound to Lyn SH2 via phosphotyrosine 299 and neighboring residues and (iii) a tyrosine phosphorylated STAT3 linked via SH2 to the regulatory, C-terminal tyrosine of Lyn. The cytoplasmic Csk kinase that negatively controls the Src kinase activity in human T lymphocytes is not involved in the B-NHL signalosome, strongly suggesting that associated Lyn and Cbp/PAG form a constitutively active signalosome in B-NHLs. An oncogenic role for Lyn was shown following exposure of B-NHL lines to small molecular weight Lyn inhibitors that prevented Lyn and Cbp/PAG phosphorylation, dissociated the signalosome from rafts and eventually induced apoptosis. Apoptotic cell death also followed decreases in Lyn or Cbp/PAG expression levels in one mantle-cell lymphoma line, but not in a Hodgkin-derived one, supporting the notion that B-NHLs are oncogenically ''addicted'' to the Lyn/PAG signalosome. The Lyn-Cbp/PAG signalosome therefore exert a proximal control on proliferation and survival in most B-NHLs, and represent a suitable therapeutic target in B-NHL cells that exhibit oncogenic ''addiction'' to the Lyn kinase.

390bis A NOVEL TRANSLOCATION T(14;16) – A RARE EVENT LEADING TO ICSBP/IRF8 OVER-EXPRESSION IN DIFFUSE LARGE B-CELL LYMPHOMA

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Introduction: In the last years, an increasing number of chromosome translocations involving the immunoglobulin heavy chain (IGH) locus on chromosome 14q32.3 have been described. Nevertheless, FISH and gene array studies revealed that there are still numerous translocations involving the IGH locus, for which the partner gene has not yet been identified. We therefore chose an LDI-PCR approach allowing the amplification of uncharacterized DNA sequences. A newly identified translocation was then further investigated on a large scale approach on tissue microarrays (TMA) by interphase FISH analysis.

Methods: Genomic DNA was extracted from frozen DLBCL. DNA was then digested with different restriction enzymes, each covering restriction sites in the joining, switch und IGG region of the IGH gene locus. Thereafter the DNA was ligated before subjecting to LDI-PCR an sequencing of the PCR products. A translocation specific interphase FISH approach was established. IHC was performed on TMA containing paraffin embedded DLBCL.

Results: In a CD5+ DLBCL we detected a new translocation (14;16) 5 kb upstream of the ICSBP/IRF8 gene on chr 16, joined to JH2 on chr 14. Consequently, ICSBP/IRF8 protein was strongly expressed in lymphoma cells of the index patient by IHC. Moreover, ICSBP/IRF8 protein was present in over 60% of a series of more than 60 DLBCLs without identifying the t(14;16) by FISH.

Conclusion: LDI-PCR is an efficient method to detect new translocations to the IGH gene in B-cell NHLs. The t(14;16) in the current case seems to be functionally involved as it up-regulates the protein expression of a transcription factor. It seems, however, to be a rare event in DLBCLs. Since neither translocation t(14;16) nor amplification of ICSBP/IRF8 could be identified in the remaining DLBCL, further mechanisms are responsible for its overexpression. With additional analysis investigating a correlation with GC and ABC phenotypes and survival, we tend to elucidate a possible role of ICSBP/IRF8 in DLBCL.

391 NON-PEPTIDIC SMALL MOLECULE INHIBITORS (SMI) OF BCL-2 FAMILY PROTEINS AS THERAPEUTIC AGENTS IN LYMPHOMA

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Introduction/Background: The Bcl-2 family of proteins is an attractive therapeutic target in lymphoma given its role in lymphomagenesis and resistance to chemotherapy. SMI mimicking the BH3 domain bind to and disarm anti-apoptotic proteins. We present here data on new BH3 mimetic SMI, TW-37 on a spectrum of B-cell tumors.

Material and Methods: The compound TW-37, N-[2-tert-butyl-benzenesulfonyl)-phenyl]-2,3,4 trihydroxy-5-(2-isopropyl-benzyl)-benzamide, was syhesized in the laboratory of S. Wang (University of Michigan). Equilibrium binding (K_D) of TW-37 to Bcl-2 was measured using surface plasmon resonance (SPR). Effect of TW-37 on growth of malignant B-cells was assessed on 4 cell lines: WSU-pre-B-ALL, WSU-DLCL2, WSU-FSCCL and WSU-WM in addition to fresh patient samples. Protein expression of Bcl-2 family proteins, caspase activation and apoptosis were determined by Western blots (WB).

Results: TW-37 inhibited cell growth and induced apoptosis on all established cell lines and fresh tumors with IC_{50} ranging between 165 nM and 320 nM. The compound binds with highest affinity to Bcl-w (1.48 nM) and lowest affinity to Bcl-2 (150-170 nM); equilibrium binding to Mcl-1 occurs at 27 nM. TW-37 competes with tBid for the binding pocket of Bcl-2. However, it was not able to disrupt an already formed Bcl-2-tBid heterodimer with TW-37 concentrations as high as 10,000 nM. WB analysis of all Bcl-2 family proteins suggested that TW-37 response is best predicted by Bax/Mcl-1 ratio which varied over 6-folds between the 4 cell lines tested. So far, fresh patient samples fit this curve.

Conclusions: SMI of Bcl-2 family proteins are effective in inducing growth inhibition and apoptosis of lymphoma cells. Such molecules must bind to their targets before heterodimers form between natural pro- and anti-apoptotic proteins. A high Bax/Mcl-1 ratio in lymphoma cells may be an important predictor of response to TW-37. This compound may stand apart from other BH3 mimetic SMIs in its ability to hit all drug targets including Mcl-1.

392 PROMOTER METHYLATION OF DAP-K, SHP1, RARβ AND P15 IN PATIENTS WITH DIFFUSE LARGE CELL LYMPHOMA (DLCL) AND BENINGFOLLICULAR HYPERPLASIA (BFH)

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Introduction: Transcriptional silencing of tumor suppressor genes, associated with DNA methylation, is an epigenetic event known to occur in leukemias, MDS and lymphoid neoplasms. Much less is known about the specific methylation changes that occur under physiological conditions such as BFH. We determined the methylation status of 4 tumor suppressor genes in 40 patients with DLCL and 20 patients with BFH. The target genes chosen are potentially involved in B-cells malignancies and encoding proteins implicated in apoptosis regulation (DAP-K), Jak/STAT3 signalling pathway (*SHP1*), hormonal response (*RAR*b) and cell cycle control (*p15*).

Material and Methods: Genomic DNA extracted from paraffin-embedded samples of 40 DLCL and 20 BFH were analyzed by methylation-specific polymerase chain reaction to determine promoter hypermethylation of DAP-k, SHP1, Rarβ and p15. DLCL samples were obtained mostly from lymph nodes. All BFH samples were obtained from lymph nodes or tonsil. Diagnosis was based on morphology and immunohistochemistry analysis. All cases were matched for age, sex and ethnic origin.

Results: DAP-k, SHP1, p14 and RARb promoters are methylated in DLCL and BFH. DAP-k promoter methylation occurred with higher frequency in DLCL (73%) than in BFH (43%) (p=0.036). Eventhough SHP1 and p14 were methylated more frequently in DLCL than in BFH (56% vs 41% and 59 vs 40%) the difference was not statistically significant (p=0.38 and p=0.2). No difference was found in RARb promoter methylation between both entities (66% vs 60%, p=0.630).

Conclusions. Inactivation of DAP-K, SHP1 and Rarβ is present in DLCL and BFH and only in the former its frequency is significantly higher in DLCL. Therefore, it may represent a physiologic event conferring a temporal survival advantage necessary for a BFH response. With our data methylation of cyclin dependent kinase inhibitors such as p14 is not a differential pathogenic event in DLCL with respect to BFH.

393 EXPRESSION OF INTRACELLULAR ABC TRANSPORTER A3 IN AGGRESSIVE NON-HODGKIN LYMPHOMA

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Introduction: Despite advances in the chemoimmunotherapy of patients with malignant lymphoma, resistance to chemotherapy still represents a significant hindrance to disease control for a subgroup of patients with aggressive lymphomas. We previously found the intracellular ABC transporter A3 (ABCA3) strongly expressed in myeloid hematopoietic malignancies, and discovered significant drug resistance to be associated with the expression of this protein.

Methods: We investigated ABCA3 expression on mRNA and protein level in a spectrum of 21 cell lines representing all major types of lymphohematopoietic malignancies, as well as cohort of samples from patients with primary aggressive lymphoma, uniformly treated with CHOP-like therapy within the German multicenter studies NHLB1, NHLB2, and MINT of the German high grade lymphoma study group.

Results: In the cell lines, RT-PCR results detected transcripts of ABCA3 in all types of hematopoietic malignancies, with highest yields in cell lines derived from patients with aggressive B-cell lymphoma disease. In cell line models, ABCA3 expression was associated with significant detoxification capacity and resistance to chemotherapy exposure. In accordance with the expression pattern in myeloid cells and normal human hematopoietic stem cells, ABCA3 protein was found in the limiting membrane of cytoplasmic lysosome-related organells. Immunohistochemistry on 240 samples of a tissue microarray confirmed the expression of ABCA3 in primary lymphoma tissue, and allowed classification in groups of low, intermediate and high expression levels based on a semiquantitative scoring system. Correlating such expression levels to the clinical end points of overall survival, disease-free survival showed no impact of ABCA3 tissue protein levels on the patients' prognosis.

Conclusion: The intracellular ABC transporter A3 is strongly expressed in aggressive lymphoma cell lines and tissues, and mediates subcellular drug sequestration. Its role for lymphoma cell biology and susceptibility to chemotherapy warrants further investigation.

394 IDENTIFICATION OF PROTEOMIC CHANGES IN SIGNALING UPON ONCOGENE INACTIVATION AFTER THERAPEUTIC TREATMENT IN HEMATOPOIETIC TUMORS USING A NOVEL NANOSCALE-IMMUNOASSAY SYSTEM

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Hematopoietc tumors are caused by the activation of oncogenes and inactivation of tumor suppressor genes. Previous reports have shown that using conditional transgenic model of MYC-induced lymphoma, inactivation of the MYC oncogene can result in sustained regression of hematopoietic tumors. Recently, we have found that the HMG-CoA reductase inhibitor, atorvastatin has potential clinical activity in patients with lymphoma. To measure the influence of this and other agents on the regulation of oncogenes and tumor suppressors and associated signaling transduction pathways in patients, we have developed a novel nano-scale immunoassay system to measure changes in expression and activation of proteins including MYC, AKT, ERK, STAT3 and STAT-5 in pre-clinical mouse models as well as human patients with lymphoma and CML before and after therapeutic oncogene inactivation. We will present clinical data illustrating that we can detect discrete proteomic changes in fine needle aspirates from patients before and after therapeutic treatment. We will present data illustrating that atorvastatin surprisingly exhibited clinical and biological activity in patients with lymphoma. Time course studies demonstrated the decrease in phosphorylation of key signaling proteins in lymphoma patients and after treatment with atorvastatin and in CML cells after imatinib treatment. A single pan-specific antibody was used to distinguish between the phosphorylated and non-phosphorylated protein isoforms, as the nanoscale-immunoassay technology separates different phosphorylated forms of a protein based on their isoelectric point. Our novel nanoscale immunoassay system can potentially be generally used for high throughput analysis of microscopic clinical specimens for the analysis of unexpected therapeutic activities of existing drugs as well as for biomarker discovery, molecular diagnostics and clinical screening during therapeutic interventions.

395 BCL-2 NEGATIVE FOLLICULAR LYMPHOMA IS ASSOCIATED WITH SOMATIC HYPERMUTATION OF THE IGV$_H$ GENES AND ABERRANT SOMATIC HYPERMUTATION

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Follicular lymphoma (FL) is typically characterized by the t(14;18) translocation resulting in the constitutive expression of bcl-2 protein, however approximately 10-15% of FLs are negative for bcl-2 protein, and a small fraction of them (~5%) does not exhibit the t(14;18) translocation either. These bcl-2 negative cases are usually grade 3 FLs, which are suggested as a distinct entity in the WHO classification of lymphomas by having different molecular and genetic characteristics that delineate them from other FLs. In this entity the role of somatic hypermutation (SHM) in the diversification of immunoglobulin genes and the malfunction of the physiological SHM, termed aberrant somatic hypermutation (ASHM), which affects additional genes such as *PAX-5, RhoH/TTF, c-Myc, PIM1,* is not explored. To further characterize the molecular pathways and the genetic alterations associated with the pathogenesis of the bcl-2 negative FLs, we have analyzed the mutational status of *c-Myc*, *PAX-5* and *RhoH* genes determining the possible role of ASHM in tumorgenesis. To explore the SHM activity of these lymphomas we investigated the mutational profile of Ig V_H genes. Tumor cells showed ongoing SHM of the Ig V_H genes. The distribution of these mutations was highly consistent with antigen selection. Mutations in one or more genes affected by ASHM were detected in the majority of bcl-2 negative FLs. Thus our results show that both SHM and ASHM are characteristic features of the bcl-2 negative FLs.

396 CORRELATION OF PLASMA VASCULAR ENDOTHELIAL GROWTH FACTOR AND SERUM INTERLEUKIN-6 WITH POOR PROGNOSTIC MARKERS AND THE CLINICAL FEATURES OF NON-HODGKIN LYMPHOMA

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Background: Many angiogenic factors including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), and interleukin (IL)-6 showed significant correlation with prognosis of solid tumors. However, the clinical implication of circulating angiogenic factors in patients with non-Hodgkin's lymphoma (NHL) has not been fully elucidated. The aim of the study is to evaluate the usefulness of circulating angiogenic factors as diagnostic and prognostic markers in patients with NHL.

Methods: Angiogenic factors including VEGF, HGF, bFGF, IL-6, VEGF receptor 1 (VEGFR1), and VEGF receptor 2 (VEGFR2) were measured in blood samples of 87 NHL patients and 131 healthy unrelated controls. Clinical characteristics such as histologic type and international prognostic index (IPI) including age, performance status, LDH, stage, extranodal involvement were evaluated. The hazard ratios (HRs) for clinical variables and circulating angiogenic cytokines were determined in terms of risk for overall survival.

Results: The baseline levels of plasma VEGF, serum VEGF, plasma bFGF, and serum IL-6 were significantly elevated in patients than controls. Plasma VEGF and serum IL-6 levels were significantly elevated according to the increment of stages and those were higher in the patients with extranodal involvement of lymphoma and those with high/high intermediate IPI. The HRs of plasma VEGF were 1.004 [95% confidence interval (CI), 1.001-1.007] in NHL.

Conclusions: This study indicated that the blood levels of angiogenic factors are associated with the clinical characteristics in patients with NHL, especially plasma VEGF and serum IL-6 can be used as prognostic markers for NHL.

397 THE ROLE AND PROGNOSTIC SIGNIFICANCE OF KI-67 INDEX IN NON-HODGKIN'S LYMPHOMA

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Introduction: An important and easy to use proliferation marker is the nuclear protein Ki-67, which is an antigen present in all proliferating cells and thus determines the growth fraction of tumors.

Material and methods: The Ki-67 proliferation index (PI) assayed immunohistochemically in tissue samples of 319 newly diagnosed NHL patients was evaluated according to the WHO lymphoma classification and NCI grade. In 268 patients the correlation between Ki-67 PI and the clinical course and survival outcome was also analyzed.

Results: There was a significant difference in mean Ki-67 PI among different lymphoma sub classifications ($p<0.001$). The mean Ki-67 PI in indolent lymphomas was 26.6%, in aggressive lymphomas 67.2% and in highly aggressive lymphomas 97.6% ($p<0.001$). A cut-off value of 45% was assessed by ROC curve (area under the curve = 0.877, $p<0.001$) as differentiating between indolent and aggressive lymphomas. It detected 82.8% of the indolent lymphomas (Ki-67 PI <45%) and 85% of the aggressive lymphomas (Ki-67 PI >45%). In patients with DLBCL (n=141), a correlation was found between low or high Ki-67 PI (cutoff at 70%) and patient age and performance status (PS). A high Ki-67 PI was found in 41.5% of younger patients (age <60) compared to 61.4% of older patients (age >60) ($p=0.025$). A high Ki-67 PI (>70%) was found in 49% of patients with good PS (0-1) compared to 69% of patients with poor PS (PS 2 and higher) ($p=0.04$).The 3 years survival of DLBCL patients with low Ki-67 PI was 75±5.6% compared with 55.9±6% in patients with high Ki-67 PI ($p=0.015$).When combining the Ki-67 PI with other prognostic factors, like the IPI score, and disease bulk, Ki-67 PI added to prognosis evaluation in the group of patients

with low IPI (≤2) and patients with disease bulk >10cm. The 3 years survival in patients with low IPI (≤2) was 94±4.1% if they had a low Ki-67 PI (<70%) and 64±8.1% if they had high Ki-67 PI (>70%), (p=0.002). Patients with bulky disease (>10cm) had a significantly better survival if they presented a lower Ki-67 PI value (3 years survival of 100% vs. 25±12%, p=0.012).

Conclusions: Different lymphoma entities have different mean Ki-67 index. A cutoff value of 45% can differentiate between indolent and aggressive lymphomas. In DLBCL a cutoff of 70% differentiated between good and bad prognosis and correlated with patient age and PS. Ki-67 PI adds to the prognosis of DLBCL patients with low IPI and patients with bulky disease.

398 RITUXIMAB-MEDIATED ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY AGAINST CLL IS ENHANCED BY INTERLEUKIN-15

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Introduction: CLL cells show a poor response to rituximab due, in part, to low expression of CD20. Antibody-dependent cellular cytotoxicity (ADCC) seems to be an important in vivo mechanism of rituximab. The activating receptor Fcg RIIIa, mostly expressed in NK cells, plays a major role in the rituximab-mediated ADCC. Interleukin-15 (IL-15) acts at different stages of the immune response by expanding and activating NK cells. We have studied the effect of IL-15 in the enhancement of rituximab-mediated ADCC against CLL cells.

Methods: ADCC was performed by standard ^{51}Cr release assays using peripheral blood mononuclear cells (PBMC) purified from 10 normal volunteers by gradient centrifugation. Cells were cultured for 24 hours in the presence of recombinant human interleukin 15 (rhIL-15; 10 ng/ml). PB (>90% tumor cells) from 10 patients diagnosed with CLL was used as target. CLL cells were incubated in the presence of rituximab (10 m g/ml) or human IgG1 as control. The polymorphism at amino acid 158 of the activating Fcg RIIIa receptor was analyzed in effector cells with a polymerase chain reaction (PCR)-based allele-specific restriction analysis assay. IFN-g secretion was quantified by ELISA.

Results: Minimal natural cytotoxicity could be detected at the highest ratio (2.7% 1.7% lysis, mean SEM of 10 independent experiments). In the presence of rituximab, PBMC from 10 donors showed low levels of ADCC (13.4% 2.5% lysis, mean SEM). However, under the same experimental conditions, when effector cells were activated with IL-15 a significant increase in the lysis of CLL cells was observed (33.2% 4.9% vs. 13.4% 2.5%, IL-15 plus rituximab vs. rituximab alone) (p<0.0001). No relationship between the percentage of lysis and the Fc RIIIa genotype was observed. IFN- production by IL-15-activated effector cells was higher than by unstimulated-effector cells (1228.4 ± 420.5 vs 217.5 ± 65.4 pg/ml, respectively; p=0.03).

Conclusion: PBMC are poor effector cells to kill CLL B cells in the presence of rituximab. Treatment of PBMC with IL-15 enhanced rituximab-mediated ADCC against CLL. These results may be useful to improve the therapeutic activity of rituximab against CLL.

399 RESPONSE TO SGN-40 IN PRE-CLINICAL NHL MODELS IS PREDICTED BY CD40 PATHWAY ACTIVATION STATUS AND GERMINAL B-CELL IDENTITY

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Introduction: SGN-40 is a humanized IgG1 monoclonal antibody that binds to CD40, mediates effector cell functions (ADCC/ADCP), and stimulates downstream signaling pathways; SGN-40 has shown activity in a phase I single agent multi-dose trial in NHL, with greatest activity in diffuse large B-cell lymphoma (21%, or 3/14 pts) (Advani et al. 2006, ASH). In vitro studies of the mechanism of activity implicated important components of the GCB phenotype, such as bcl-6 (Lewis et al. 2007, ASH). To identify candidate predictive markers for SGN-40 activity we determined molecular profiles in a panel of 31 NHL cell lines and correlated results with sensitivity to SGN-40 in vitro.

Materials and Methods: Differentially expressed genes were determined by a moderated t-test and Spearman's Rank Correlation using SGN-40 IC25 values. Gene Set Enrichment Analysis (GSEA) was implemented to facilitate the relationship of genes to biological function. A classifier algorithm was developed by Stepwise Linear Modeling to generate a SGN-40 Index value.

Results: GSEA revealed a substantial enrichment of genes that are regulated by CD40L stimulation. In addition, classifying DLBCL cell lines into Activated B-Cell (ABC) or Germinal Center B-Cell (GCB)-like subclasses by gene expression revealed a striking enrichment of SGN-40 activity in GCB-like cell lines. We developed a 14-gene signature utilizing genes from the CD40 pathway activation and GCB gene sets; the classifier gave >96% accuracy (30/31) on the 'training' set of cell lines and 75% (3/4) accuracy on a 'test' set of xenografts.

Conclusions: NHL cell lines exhibit differential sensitivity to SGN-40 that correlate with GCB classification and, independently, with a gene signature indicating absence of CD40 pathway activation. Based on these results, we hypothesize that SGN-40 may be most effective in NHL tumors characterized by a quiescent CD40 pathway, where activation of the CD40 pathway will trigger apoptosis. Future studies will test this hypothesis by correlating clinical response after treatment with SGN-40 with pre-treatment molecular profile determined from tumor tissue.

400 IGV$_H$ GENE MUTATION STATUS AND GENOMIC IMBALANCES IN CHRONIC LYMPHOCYTIC LEUKAEMIA WITH INCREASED PROLYMPHOCYTES (CLL/PL)

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Chronic lymphocytic leukaemia (CLL) with increased prolymphocytes (CLL/PL) has been defined by the World Health Organization (WHO) classification and considered as a progressive and clinically aggressive variant of CLL. To further characterize the biological features of this disease, we performed IgV$_H$ gene mutational status, FISH and high resolution comparative genomic hybridization (HR-CGH) analysis in 17 cases of CLL/PL. All CLL/PL utilized members of V$_H$1, V$_H$3 and V$_H$4 families, with the highest prevalence of the V$_H$1-69 gene. In all but one cases analyzed, the V$_H$ genes were unmutated. The FISH and HR-CGH analyses showed frequent occurrence of trisomy 12, del(11)(q23), del(17)(p13) and genetic imbalances, but recurrent genetic lesion characteristic for CLL/PL was not found. The follow-up HR-CGH analysis of two cases showed that increase of prolymphocytes in the course of CLL or CLL/PL are associated with clonal evolution and selection of the tumour clone. In conclusion, this study suggests that CLL/PL is a relatively homogeneous disease regarding V$_H$ gene mutation, but heterogeneous regarding genetic lesions. The heterogeneity and high number of genomic imbalances found in CLL/PL suggest that a genome-wide instability of the neoplastic cells may play a role in the development of the disease.

401 NK-S1, AN NK/T NON-HODGKIN'S LYMPHOMA XENOGRAFT FOR DRUG TESTING AND THERAPEUTIC INTERVENTION

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NK/T non-Hodgkin's lymphomas (NHL) are more frequently observed in Asia, South and Central America compared with the United States and other Western populations. Disseminated NK/T NHL is mostly incurable and responds poorly to conventional anthracycline-based regimens that are used to treat B cell NHLs. We have established an EBV positive NK lymphoma xenograft (NK-S1) in SCID mice, from the testicular metastasis of a patient who presented with nasal-type NK lymphoma. The xenograft retained the same immunophenotypic features of the original, and was positive for cytoplasmic CD3, TIA-1, granzyme B and EBER. This xenograft has been passaged exclusively *in vivo*. We have previously tested and found doxorubicin to have no effect on the growth pf subcutaneous NK-S1 xenografts. In this study, we found that both IP gemcitabine (60mg/kg q3d x2 and q7dx4) and IV oxaliplatin (q7d x3) caused complete remission of the tumors. However at D39 after treatment was first instituted, tumour re-growth was observed. IP campath (200mg/dose q3d x10) also caused tumours to go into CR but in contrast, no tumour re-growth was observed up to 2 months since the first dose was administered. In conclusion, NK-S1 xenograft appears to be a useful *in vivo* platform for preclinical drug testing.

402 SELECTIVE INDUCTION OF APOPTOSIS IN HUMAN B LYMPHOMA CELLS BY THE ETHER LIPID ET-18-OCH3 (EDELFOSINE): INVOLVEMENT OF CALCIUM SIGNALLING

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Ether lipids represent a novel class of promising antitumor agents and some of them have attracted great attention due to their antineoplastic activities. Thus, several of these compounds are scheduled for, or currently undergoing Phase I/II clinical evaluation. One of the most encouraging and studied of the ether lipids is edelfosine. It is a potent inducer of apoptosis in tumor cells sparing normal cells. However, the mechanisms underlying its effects remain to be elucidated. It has been recently shown to modulate cell death through recruitment of Fas/CD 95 death receptor, FADD and procaspase 8 in lipid rafts in hematologic malignancies and through an endoplasmic reticulum stress in solid tumor cells. Fas/CD 95 death receptor, lipid rafts, and ER stress being linked to calcium signalling, we have studied edelfosine effects on Ca^{2+} signalling (microfluorimetry using Indo 1 as Ca^{2+} indicator) in B lymphoma cells and B lymphocytes isolated from children tonsils. Edelfosine has a dose-dependent effect on intracellular Ca^{2+} concentration in B lymphoma cells and a reduced effect in normal B lymphocytes. Edelfosine-induced intracellular calcium increases predominantly resulted from stimulations of Ca^{2+} influx through nickel-sensitive calcium channels. In

parallel, edelfosine has the same dose dependent effects on apoptosis (DYm measurements using TMRM as mitochondrial transmembrane potential indicator). Furthermore, chelation of intracellular Ca^{2+} ions with BAPTA-AM strongly reduced edelfosine-induced apoptosis. These data confirm the selective effect of edelfosine in a wide spectrum of transformed cells and suggest the involvement of Ca^{2+} signalling in edelfosine-induced apoptosis. In addition, edelfosine augments the size of the Ca^{2+} mitochondrial store. This could result in the formation of permeability transient pores, the release of cytochrome *c* into the cytoplasm, and apoptosis. Due to its specific action on cancer cells, edelfosine is being used as an anticancer drug in myelomas, sarcomas, and prostate cancers. Our works show that this ether lipid could also have a therapeutic interest in the treatment of B lymphoma, only or in association with chemotherapy.

403 EVIDENCE OF SGN-40-INDUCED CHEMOSENSITIZATION OF NON-HODGKIN'S LYMPHOMA TUMORS

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Background: SGN-40 is a humanized monoclonal antibody that targets CD40, triggers pro-apoptotic signal transduction, and mediates effector cell function (ADCC/ ACDP). SGN-40 overcomes rituximab resistance and improves chemotherapy efficacy in xenograft models and has been shown to up-regulate the p53 family member TAp63α, a chemo-sensitizing transcription factor capable of inducing apoptosis when combined with cytotoxic agents.

Methods: Observational case series based on medical records review of patients (N=4) treated with gemcitabine immediately after progression on single-agent SGN-40. Patients received SGN-40 as participants on a clinical trial of SGN-40 in relapsed/ refractory NHL.

Results: *Patient 1:* 72 YO female with DLBCL, who previously failed multiple therapies (R-CHOP, ESHAP, lenalidomide, gemcitabine). The patient was enrolled on the SGN-40 study. After completion of Cycle 1 of SGN-40, progression was documented (largest tumor a 12.3 x 6.8 cm left inguinal mass). Three days after the last dose of SGN-40, gemcitabine was started at a reduced dose due to thrombocytopenia (600 mg/m^2 on days 1, 8 and 15). After 2 months of therapy, CT scans demonstrated a PR (58% reduction) which lasted 7 months. *Patient 2:* 42 YO female with DLBCL failed multiple therapies (R-CHOP, RT, ASCT) with PR as best response. Following 1 cycle of SGN-40, progression was documented (largest tumor a 11.3 × 5.1 cm periumbilical soft tissue mass). Two days after the last dose of SGN-40, gemcitabine was started (1000 mg/m^2 on days 1, 8 and 15). After 2 months of therapy, CT scans demonstrated a CR in all sites of disease. Response lasted 12 months at which time relapse was documented in the uterus. *Additional Patients:* two additional patients were treated with gemcitabine immediately following SGN-40, both achieved a PR.

Conclusions: Although these clinical observations were made outside of a controlled trial, the dramatic responses observed in these heavily pretreated patients suggest that SGN-40 may sensitize tumor cells to chemotherapy. These observations warrant further evaluation in clinical trials of SGN-40 in combination with gemcitabine and/or other chemotherapy regimens for the treatment of patients with NHL.

404 MORPHOIMMUNOHISTOCHEMICAL AND CLINICAL CHARACTERISTICS OF FOLLICULAR LYMPHOMA GRADE III

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Introduction: Approaches to treatment follicular lymphoma grade III (FL III) represent a difficult clinical problem. Now calls in question validity of inclusion FL III in group of an intermediate grade.

Material and methods: FL III is established at 20 pts (22-78, median - 46 years) with using morphological and immunohistochemical methods.

Results: At morphological research the heterogeneous picture is revealed. So, FL IIIA consists of patterns FL II in 35% of cases. Combination FL IIIB and DLBCL is established in 5% cases. P53 is positive in 75% of cases (threshold of an estimation >10% of positive cells). The high level of proliferative activity (Ki-67 >50%) is demonstrated at 20% of pts. According to criteria FLIPI the group of low risk includes 22% of pts, 21% pts are referred to group of intermediate risk, the group of high risk - 57% of pts. The involvement of bone marrow is observed at 28% of pts.

Conclusion: FL III (A, B) represents the stage of tumor progression, does not arise de novo. Risk of transformation FL III in DLBCL is high using criteria Ki-67, P53 and different patterns of morphological pictures. FL III demands the treatment under the programs developed for DLBCL.

405 AGE RELATED EPSTEIN BARR VIRUS ASSOCIATED B-CELL LYMPHOPROLIFERATIVE DISORDER: REPORT OF NINE PERUVIAN CASES

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Background: Age related Epstein Barr virus B cell associated Lymphoproliferative Disorder is a new entity with aggressive course and poor survival. Oyama et al. described this new entity in 96 Japanese patients.

Patients and Methods: Nine Peruvian patients, diagnosed as having Age related EBV associated B cell Lymphoproliferative disorder were included in the report. All patients were positive for EBER using the in situ hybridization (ISH). Immunohistochemical expression of CD20, BCL6, CD10, and MUM-1/IRF4 was examined using a tissue microarray (TMA) technique.

Results: mean age was 70 years old (range 31-85). Only one patient had less than 65 years old. Male/female ratio was 5/4. B symptoms occurred in 5/9. Zubrod >1 in 8/9 patients. Advanced stage (III/IV) in 8/9 patients. IPI more than 2 in 8/9 patients. Extranodal involvement was: pleura (n=2), suprarenal gland (n=1), stomach (n=1) and bone marrow (n=1). Morphology in all cases was large cell lymphoma. All patients had a non germinal center like phenotype. Four patients died before treatment and five received CHOP regimen. Two had complete response and three had progression disease. All cases died during first year and only one patient had a long survival (42 months).

Conclusions: Age related EBV associated B cell Lymphoproliferative disorder is a new entity, related to more advanced age, poor status performance, non-germinal center like phenotype and very short overall survival.

406 DETECTION OF DIFFERENT CLONAL EBV STRAINS IN HODGKIN LYMPHOMA AND NASOPHARYNGEAL CARCINOMA TISSUES FROM THE SAME PATIENT

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Introduction: The ubiquitous herpesvirus Epstein-Barr virus is linked to the development of several malignancies, including nasopharyngeal carcinoma (UNCT) and Hodgkin lymphoma (HL). However, the underlying mechanism for EBV-related malignancies has not been fully elucidated.

Material and Methods: In this study, we report the case of a patient with sequential development of UCNT and HL.

Results: A 29 year-old man was referred for nasal obstruction and cervical lymphadenopathy. Histologic examination of a lymph node concluded to nodal involvement by a nasopharyngeal carcinoma of the UCNT type. Tumour cells expressed the LMP1 protein. The patient was treated by three courses of chemotherapy, followed by radiotherapy on nasopharyngeal and cervical areas. Evaluation after the therapeutic procedure concluded to a complete remission. Eight years later, the patient presented a centimetric mental adenopathy. Histologic examination of this adenopathy showed a HL of mixed cellularity type. Reed-Sternberg cells expressed the LMP-1 protein. The patient was treated by four courses of modified ABVD chemotherapy without radiotherapy. TEP scan after therapy showed a complete response. After a follow-up of 30 months, the patient is still in complete remission. DNA was extracted from the paraffin blocks of both tumours and was subjected to PCR protocols for detecting *LMP-1* gene polymorphisms. We noted that HL samples had no 30 bp deletion whereas the UCNT sample was infected by a strain with a 30 bp deletion in its *LMP-1* in gene. These results clearly showed that the two tumours were infected by different viral strains.

Conclusions: The present report, which describes sequential development of HL and UCNT in one patient, seems exceptional. Our observation confirms the possibility of multiple EBV infections in a same individual. The two tumours contained different clonal viral genomes, suggesting a central and specific role of EBV strain infection in their pathogenesis.

407 BOVINE LEUKEMIA VIRUS AND HUMAN DETECTION OF ANTIBODY AGAINST BOVINE LEUKEMIA VIRUS

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Introduction: Bovine Leukemia Virus (BLV) is an oncogenic retrovirus that causes B cell leukosis in 1-5% of infected cattle. Most infected cattle do not actually develop leukemia but remain healthy and are not removed from the herd. BLV-infected cellsare present in marketed beef and dairy products thus consumption of unpasteurized dairy products or undercooked beef could possibly allow transmission of infectious virus to humans. BLV infection is not limited for cattle; the virus can infect sheep and nonhuman primates experimentally and cause cancer in the sheep in the laboratory and it can infect the cells of man species including humans and other primates. Researchers at the University of California, Berkeley found that a significant proportion of the American public may be harboring antibodies to Bovine Leukemia Virus, which they may have been exposed to through the consumption of beef or dairy products. Researchers found a disturbing trend. They found that geographically, the areas of highest cattle BLV infection did indeed seem to have significantly higher human leukemia rates.

Material and methods: This study was designed to see if any humans with acute lymphocytic leukemia (ALL) at all had antibodies to BLV. We propose here a pilot study to examine the first aspect of the overall proposal, whether humans can become infected with BLV. In this study, serum samples of fifty humans with ALL were examined for antibodies to BLV by commercial ELISA test and also for anti gp51 antibodies by AGID test.

Results: We didn't find antibodies reactive specifically against BLV in people studied with ELISA test but one case showed precipitation line in AGID test.

Conclusions: Since AGID test is not a specific test, this study failed to reveal a strict relationship between BLV and human. However, we suggest that further studies in this area could be important, by the use of more sophisticated methods.

Key words: Bovine viral leukosis, Human, Cow.